AWARD NUMBER: W81XWH-15-1-0003

TITLE: In-Depth Analysis of Citrulline-Specific CD4 T Cells in Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR: Jane Buckner, MD, Benaroya Research Institute

CONTRACTING ORGANIZATION: Benaroya Research Institute at Virginia Mason
                                                Seattle, WA 98101

REPORT DATE: January 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

                              Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;

                              Unlimited Distribution

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
January 2018	Annual	10 Dec 2016 - 9 Dec 2017

4. TITLE AND SUBTITLE
In-Depth Analysis of Citrulline-Specific CD4 T Cells in Rheumatoid Arthritis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0003

5c. PROGRAM ELEMENT NUMBER

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command Fort
Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to test the hypothesis that cit-specific CD4 T cells present in rheumatoid arthritis (RA) patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identify novel therapeutic targets for the treatment of RA. In Year 3, we have continued to make significant progress for all our goals. For Aim 1, our findings suggest that the dominant autoantigen driving disease may differ between individuals and that the character of the inflammatory response in RA may be linked to the antigen during the CD4 T cell response. Furthermore, the dominant autoantigen driving disease ultimately may determine the response to therapy. For Aim 2, our analysis of the whole blood RNA sequencing dataset suggest that T cell exhaustion is dysregulated in subjects carrying the HLA-DRB1 alleles associated with increased risk of RA. A twelve-month no-cost extension was recently approved to allow us to do additional bioinformatics analysis to strengthen these results for publication. The no-cost extension was also approved for additional time to complete both the RNA sequencing of antigen-specific T cells in Aim 2 and the ex vivo tetramer analysis of the samples from the longitudinal cohort in Aim 3.

15. SUBJECT TERMS
Rheumatoid arthritis; CD4 T cells; citrulline; HLA class II tetramers; RNAseq; Transcriptional profiling

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			
U	U	U	U	34	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Rheumatoid Arthritis (RA) affects over 1.3 million Americans. It is a chronic disease, which if untreated results in pain and permanent disability. Our current approaches to treatment are expensive, lead to systemic immune suppression and do not cure the disease. It is now known that joint-associated proteins are biochemically altered by inflammation and that these alterations provoke cellular immune responses against joint tissue. In particular, T cell responses directed against the joints drive development of RA, but are not well understood. Our research group has developed the ability to identify and isolate joint specific T cells from the blood of RA patients using a tool called HLA class II tetramers. In this proposal our objective is to use this tool to better understand the unique features of joint specific T cells and how these features change with disease activity and with therapy. The information will be useful to diagnose RA earlier – which could allow for earlier intervention, decreasing the morbidity of disease. Further it may be a means to predict response to therapy very soon after the initiation of a new therapy, which would decrease the expense and exposure to drugs that are unhelpful or potentially harmful. Findings from our DoD funded work will not only enhance our scientific knowledge related to the causes of RA, but also identify new determinants which can be therapeutically targeted while protecting the remaining immune cells needed for the patient's health.

2. KEYWORDS

Rheumatoid arthritis; CD4 T cells; citrulline; HLA class II tetramers; RNAseq; Transcriptional profiling

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goal of this project is to test the hypothesis that cit-specific CD4 T cells present in RA patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identify novel therapeutic targets for the treatment of RA. In Specific Aim 1, we will utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity. In Specific Aim 2, we will utilize C1 Fluidigm technology combined with RNAseq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects. In Specific Aim 3, we will utilize informative cell surface markers and transcript profiles to determine the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients.

Please note a twelve-month no-cost extension was approved on November 29, 2017 (Modification #1). Table 1 lists the Major Tasks and Milestones associated with each Specific Aim as outlined in the approved Statement of Work (SOW), and includes actual completion dates or percent complete. Table 2 is a projected timeline for the twelve-month no cost extension period.

TABLE 1: MAJOR GOALS, MILESTONES, TIMELINE AND COMPLETION DATES

Specific Aim 1: Utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity.	**Projected Timeline**	**Year 3 Progress**
Major Task 1: Recruit patients and conduct studies to characterize T cells that recognize citrullinated epitopes by direct ex vivo tetramer staining.	**Months**	**Completion Dates (or % Complete)**
Subtask 1: Submit documents for local IRB review.	1-2	**100% Complete** BRI IRB approved: 07/28/2014 VA IRB approved: 09/24/2014
Subtask 2: Submit IRB approval and necessary documents for HRPO review.	3-4	**100% Complete**
Milestone #1: HRPO approval received	4	**100% Complete** BRI HRPO approved: 12/24/2014 VA HRPO approved: 03/27/2015
Subtask 3: Recruit at least 20 RA subjects in each of the four disease activity groups as defined by RAPID3 score as well as 20 healthy control subjects.	4-15	**100% Complete**
Subtask 4: Ex vivo tetramer analysis of citrulline reactive T cells.	4-15	**75% Complete**
Milestone #2: Successful comparison of the frequency and phenotype of cit-specific T cells in RA subjects based on RAPID3 score categories. Submission of these data as an abstract at a national meeting.	15-16	**100% Complete** Presentations included in Appendix II.
Specific Aim 2: Utilize C1 Fluidigm technology combined with RNAseq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects.		
Major Task 2: Sorting of tetramer sorted cit-specific T cells and transcript profiling.	**Months**	**Completion Dates (or % Complete)**
Subtask 1: Preliminary Fluidigm C1 analysis of citrulline specific CD4 T cells in 2-4 RA subjects and healthy controls known to have high T cell frequency.	6-15	Whole Blood RNAseq: 90%
Subtask 2: Confirmation of RNA seq transcript signatures using qPCR of the same amplified cDNA samples.	16	20% Complete
Subtask 3: Further validate C1 findings on new or frozen PBMC samples using 96 well PCR analysis and/or flow cytometry.	17	0% Complete
Subtask 4: Select and re-sample (if needed) previously identified Tmr+ RA and healthy control subjects for transcript analysis.	17-20	50% Complete
Subtask 5: Transcript analysis and flow cytometric assessment of the significance of RA specific transcript markers in cit-specific T cells in population identified in Subtask 4.	20-24	0% Complete

Milestone #3: Co-author manuscript on the frequency, phenotype, and transcript profile of cit-specific T cells in RA subjects.	24	50% Complete
Major Task 3: Longitudinal study of the immune phenotype of cit-specific T cells in RA patients following first administration of biologic or non-biologic therapy.	**Months**	**Completion Dates (or % Complete)**
Subtask 1: Recruitment of patients for longitudinal studies.	16-26	100% Complete
Subtask 2: Selection of informative panel of markers for longitudinal studies.	24	100% Complete
Subtask 3: Longitudinal study of the immune phenotype of cit-specific T cells in RA patients.	24-32	10% Complete
Subtask 4: Data analysis / correlation of informative phenotypic markers with response to therapy	32-34	0% Complete
Milestone #4: Co-author manuscript on the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients	34-36	0% Complete

TABLE 2: PROJECTED TIMELINE FOR NO-COST EXTENSION PERIOD

	Projected Timeline In Months for NCE in Year 4
Specific Aim 1: Utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity.	
Subtask 4: Ex vivo tetramer analysis of citrulline reactive T cells.	37-39
Specific Aim 2: Utilize C1 Fluidigm technology combined with RNAseq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects.	
Subtask 1: RNA seq analysis of bulk sorted citrulline specific CD4 T cells in 2-4 RA subjects and healthy controls known to have high T cell frequency.	37-39
Subtask 2: Confirmation of RNA seq transcript signatures using qPCR of the same amplified cDNA samples.	40-42
Subtask 3: Further validate RNA-seq findings on new or frozen PBMC samples using 96 well PCR analysis and/or flow cytometry.	43-45
Milestone #3: Co-author manuscript on the frequency, phenotype, and transcript profile of cit-specific T cells in RA subjects.	48
Major Task 3: Longitudinal study of the immune phenotype of cit-specific T cells in RA patients following first administration of biologic or non-biologic therapy.	
Subtask 3: Longitudinal study of the immune phenotype of cit-specific T cells in RA patients.: Ex vivo tetramer assays and RNA-seq	37-42
Subtask 4: Data analysis / correlation of informative phenotypic markers with response to therapy	42-45
Milestone #4: Co-author manuscript on the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients	48

What was accomplished under these goals?
Specific Aim 1: Utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity.
For this Aim, we have completed *ex vivo* tetramer assays for 80 RA subjects and 30 healthy control subjects matched for age and gender. All subjects were DRB1*04:01, and all RA subjects were CCP positive. The HLA-DRB1*04:01 tetramer panel included tetramers that detect the citrullinated epitopes in the following synovial proteins: aggrecan, cartilage intermediate layer protein (CILP), enolase, fibrinogen and vimentin. Influenza tetramers were included as a control. Although data analysis is ongoing, we have already made a number of novel observations. We found that overall there is an increase in the frequency of CD4 T cells specific for citrullinated peptides derived from synovial antigens in RA subjects compared to healthy controls (**Appendix I: Figure 1A**), but a broad heterogeneity among the RA subjects with respect to overall frequency and the specificities of Tmr+ cells. In addition, both the frequency and the immunophenotype of the CD4 memory T cells, differed amongst different antigens (**Appendix I: Figure 1B and Figure 2**), this was true between and within individuals. Within this RA cohort, we also found that the frequency of CD4 T cells targeting the joint were responsive to biologic therapies targeting TNF-alpha in RA subjects with disease duration less than five years (**Appendix I: Figure 3**). Interestingly this was most pronounced for T cells that were specific for aggrecan, vimentin, and fibrinogen (data not shown). Together these findings suggest that the dominant

autoantigen driving disease may differ between individuals, and that the character of the inflammatory response in RA subjects may be linked to the antigen driving the CD4 T cell response. Furthermore, the dominant autoantigen driving disease ultimately may determine the response to therapy. As per **Milestone #2**, these data have been presented both nationally at the 2017 American College of Rheumatology (ACR) Annual Meeting (**Meeting Abstract included in Appendix II**) and internationally at the International Forum for RA 2017 (**Meeting Program included Appendix II**). In addition, we have recently submitted a manuscript describing aggrecan as an autoantigen in RA to the journal Arthritis and Rheumatology. This work was also presented at the 2016 ACR Annual Meeting **(Meeting abstract included in Appendix II**).

In the past year, we have continued our development and validation of HLADRB1*04:01 tetramers recognizing citrullinated tenascin C. Specifically, we have found that T cells recognizing citrullinated tenascin-C peptides are present in HLA-DRB1*0401 patients with rheumatoid arthritis. This work was presented at FOCIS 2017, the annual meeting for the Federation of Clinical Immunology Societies **(Meeting abstract included in Appendix II**).

Specific Aim 2: Utilize C1 Fluidigm technology combined with RNA-seq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects
In the past year, we have focused on data analysis of the whole blood RNA-seq dataset and anticipate completing the bioinformatics analysis by the end of the first quarter of 2018. For the transcriptional profiling of antigen-specific T cells, we have been using the ex vivo tetramer analysis in the cross-sectional cohort (Aim 1) to help us identify the best samples to study. Based on this work, we propose to analyze citrullinated-specific T cells that target both aggrecan and enolase. We have chosen to focus on these antigens due to our observation that these two antigen-specific T cell populations are unique with regard to frequency and immunophenotype in RA (**Appendix I: Figures 1 and 2**). We anticipate completing the RNA-seq by the end of the first quarter in 2018 with data analysis and confirmation in an independent cohort by the end of the third quarter.

Specific Aim 3: Utilize informative cell surface markers and transcript profiles to determine the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients.
Patient recruitment for this longitudinal study is complete. The treatment groups approved in the Year 2 Progress Report are outlined in **Table 1 in Appendix I**. Note for this study, we plan to include tenascin C in the HLA-DRB1*04:01 tetramer panel based on our recent work demonstrating that this is an important autoantigen in RA. We anticipate completing the *ex vivo* tetramer assays for this cohort by the end of the first quarter of 2018 with the data analysis completed by the end of the second quarter. The RNA-sequencing will occur during the second quarter with analysis complete by the end of the third quarter of 2018.

What opportunities for training and professional development has the project provided?
This project has provided opportunities for training and professional development for the following personnel: Jing Song, Virginia Muir and Cliff Rims. Dr. Song, a postdoctoral fellow in Dr. Buckner's lab, has been actively involved in the expansion of our tetramer panels. Her work has shown that T cells that recognize citrullinated tenascin-C peptides are present in HLA-DRB1*0401 patients with rheumatoid arthritis. She has regularly presented her work at BRI and had the opportunity to give an oral presentation at the 2017 FOCIS Annual Meeting in Chicago. She is currently preparing a manuscript based on the Tenascin C study. Dr. Muir, a postdoctoral fellow in Dr. Linsley's group, is using bioinformatics and systems immunology to analyze both the tetramer data and the whole blood RNA-seq data from the cross-sectional cohort. She regularly presents her work at BRI, and will be the lead author on any of the manuscripts based on this work. Cliff Rims, a research technician in Dr. Buckner's lab, has been actively involved in the tetramer analysis of the cross-sectional cohort. He had the opportunity to give an oral presentation based on this work at the 2017 ACR Annual Meeting in San Diego.

How were the results disseminated to communities of interest?
In the past year, we have presented our findings at the FOCIS Annual Meeting in Chicago and the ACR Annual Meeting in San Diego and at the International Forum for RA in Stockholm, Sweden. We have continued to share our findings and technology with our collaborators at both the Karolinska University in Stockholm,

Vivianne Malmstrom and Lars Klareskog, and the University of Colorado in Denver, Michael Holers. We have also worked with the Accelerated Medical Partnership RA project group, using the tetramers to evaluate synovial T cells isolated from joint biopsies that are being obtained by this research group. Collectively, these presentations demonstrate that we are regularly disseminating our results to the national and international Rheumatology Community. Furthermore, the tools generated in this project will be made available to the Rheumatology Community for the application to questions and sample sets beyond the scope of our DOD project.

What do you plan to do during the next reporting period to accomplish the goals?
We plan to complete Milestones #3 and #4 by the end of the twelve-month no-cost extension period. For Milestone #3, we will submit a manuscript describing our novel finding that the dominant autoantigen driving disease differs between individuals. We will also intend to submit a second manuscript describing our finding that dysregulated T cell exhaustion is associated with HLA-DRB1 alleles associated with risk of RA. For Milestone #4, we will submit a manuscript on the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients.

4. IMPACT
What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS
Nothing to Report

Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals.
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

6. PRODUCTS
Publications, conference papers, and presentations

1. **2017 FOCIS Annual Meeting Abstract Number: T.18.**
   Identification and Functional Characterization of T cell Reactive to Citrullinated Tenascin-C in HLADRB1* 0401-Positive Rheumatoid Arthritis Patients
   Jing Song, Cliff Rims, David Arribas-Layton, Eddie James and Jane Buckner
   Benaroya Research Institute at Virginia Mason, Seattle, WA
   Abstract included in Appendix II

2. **2017 ACR/ARHP Annual Meeting Abstract Number: 951**
   Cross Sectional Analysis of Citrullinated-Synovial Antigen-Specific CD4+ T Cells in an RA Cohort Demonstrates Antigen Based Differences in T Cell Frequency, Phenotype and the Influence of Immunotherapy
   Cliff Rims[1], Sylvia Posso[1], Bernard Ng[2], Jeffrey Carlin[3], Eddie James[4] and Jane H. Buckner[4], [1]Translational Research, Benaroya Research Institute at Virginia Mason, Seattle, WA, [2]Rheumatology, VA Puget Sound Healthcare System, Seattle, WA, [3]Rheumatology, Virginia Mason Medical Center, Seattle, WA, [4]Benaroya Research Institute at Virginia Mason, Seattle, WA
   Abstract included in Appendix II

3. **2017 International Forum for RA (IFRA) Program**
   Session 7: Adaptive immunity vs. innate immunity and mesenchymal functions in RA
   Genetics, T cell specificity and T cell regulation in RA
   Jane Buckner, Benaroya Research Institute, Seattle, WA
   Meeting Program included in Appendix II

Journal publications
Nothing to Report

Books or other non-periodical, one-time publications.
Nothing to Report

Other publications, conference papers, and presentations
Nothing to Report

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Biospecimen collections: In the past year, through recruitment of RA subjects for this study, we have made a significant contribution to the BRI Immune Mediated Disease Registry and Repository (BRI-IMDR). Specifically the number of RA subjects in the BRI-IMDR has increased from 738 to 826. The samples collected from these subjects will be first used to address questions related to the DOD project, but remaining samples will be available to other scientists for their investigation into the causes of immune-mediated disease.

Research material: We have also developed a panel of HLA-DRB*04:04 tetramers. This new tool allows us to characterize T cell responses in patients with RA and healthy subjects with the DDRB1*04:04 haploytpes.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	Jane Buckner, MD
Project Role:	Principal Investigator
Research Identifier (e.g. ORCID):	JBUCKNER
Nearest person month worked:	1
Contribution to project:	Dr. Buckner will direct the research, supervise the postdoctoral fellow and research technicians in this project. She will meet with all investigators on a monthly basis and be responsible for preparation of publications.

Name:	Bernard Ng, MD
Project Role:	Principal Investigator
Research Identifier (e.g. ORCID):	BERNARDNG
Nearest person month worked:	1
Contribution to project:	Dr. Ng will supervise recruitment of study participants at the Seattle VA.

Name:	Eddie James, PhD
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID):	EJAMES2
Nearest person month worked:	1
Contribution to project:	Dr. James will work closely with Dr. Buckner and her team on Aim 1 applying the myc–tagged tetramer technology and multiparameter flow cytometry to RA samples. Dr. James will assist in analysis of these data and preparation of publications.

Name:	Peter Linsley, PhD
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID):	PLINSLEY
Nearest person month worked:	1
Contribution to project:	Dr. Linsley will direct the RNAseq studies, and oversee the work of biostatisticians analyzing the data. He will also assist with data interpretation and preparation of publications.

Name:	Jing Song
Project Role:	Postdoctoral Fellow
Research Identifier (e.g. ORCID):	
Nearest person month worked:	6
Contribution to project:	Dr. Song will work with Drs. James and Linsley for the tetramer analyses and RNAseq studies.

Funding Support:	National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Allergy and Infectious Diseases, Benaroya Research Institute at Virginia Mason

Name:	**Virginia Muir**
Project Role:	Postdoctoral Fellow
Research Identifier (e.g. ORCID):	
Nearest person month worked:	1
Contribution to project:	Dr. Muir will work with Dr. Linsley on the RNAseq studies.
Funding Support:	National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Allergy and Infectious Diseases, Benaroya Research Institute at Virginia Mason

Name:	**Cliff Rims**
Project Role:	Research Technician
Research Identifier (e.g. ORCID):	
Nearest person month worked:	2
Contribution to project:	Mr. Rims will assist Dr. James and Dr. Buckner in handling blood samples, FACs staining and tetramer analysis.

Name:	**Jeffrey Carlin, MD**
Project Role:	Director of the BRI Rheumatic Disease Registry
Research Identifier (e.g. ORCID):	JSCARLIN
Nearest person month worked:	1
Contribution to project:	Dr. Carlin is the director of the rheumatic disease registry at BRI. He oversees patient recruitment at BRI-Virginia Mason Medical Center.
Funding Support:	Virginia Mason Medical Center

Name:	**Sylvia Posso**
Project Role:	Clinical Research Coordinator
Research Identifier (e.g. ORCID):	
Nearest person month worked:	0
Contribution to project:	Ms. Posso is the clinical research coordinator responsible for patient recruitment, maintaining IRB approval and clinical data management at BRI-Virginia Mason Medical Center.
Funding Support:	Benaroya Research Institute at Virginia Mason internal funding

Name:	**Kaytlyn Ly**
Project Role:	Research Assistant
Research Identifier (e.g. ORCID):	
Nearest person month worked:	10
Contribution to project:	Ms. Ly is responsible for patient recruitment at BRI-Virginia Mason Medical Center.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
See Appendix III, Senior/Key Personnel Other Support

What other organizations were involved as partners?

Organization Name: Seattle Institute for Biomedical & Clinical Research
Location of Organization: 1100 Olive Way, Seattle WA 98101
Partner's contribution to the project: Collaboration

The Seattle Institute for Biomedical & Clinical Research receives and administers non-VA appropriated funds in support of research performed at the VA Puget Sound Health Care System. The collaborating organization is the Seattle Institute for Biomedical & Clinical Research. The performance site is the VA Puget Sound Health Care System.

Partnering Organization Performance Site:
Department of Veterans Affairs
Puget Sound Health Care System
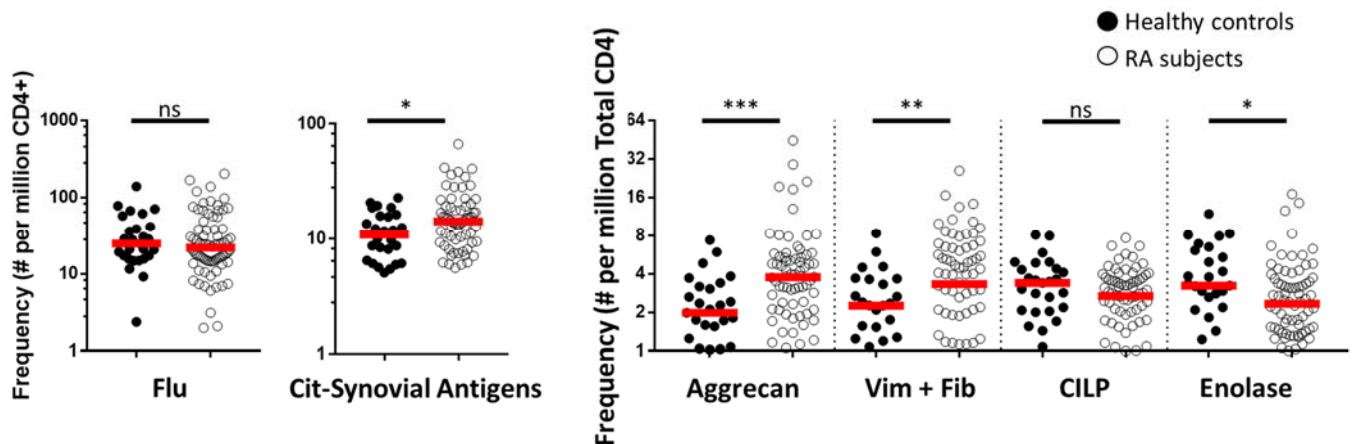1660 S. Columbian Way
Seattle, WA 98108-1597

8. SPECIAL REPORTING REQUIREMENTS
The collaborating Principal Investigator's (Bernard Ng) technical report is a duplicate that is separately submitted.

Appendix I: Figures and Tables

Figure 1. The frequency of CD4 T cells specific for citrullinated synovial antigens in RA subjects compared to healthy controls. Samples were assessed for frequency of Tetramer + CD4 T cells specific for citrullinated Aggrecan, α-Enolase, CILP, Vimentin and Fibrinogen With Influenza used as a control **A)** The frequency of CD4 T cells specific for citrullinated synovial antigens is increased significantly in RA subjects compared to controls. Data shown is combined for all tetramers targeting synovial antigens. In contrast, the frequency of influenza specific CD4 T cells is not significantly different between RA subjects and healthy controls. **B)** The frequency of both aggrecan-specific and vimentin + fibrinogen-specific T cells are significantly increased in RA subjects compared to controls. In contrast, enolase-specific T cells are decreased significantly in RA subjects. Unpaired non-parametric Mann-Whitney test * p-value <0.05
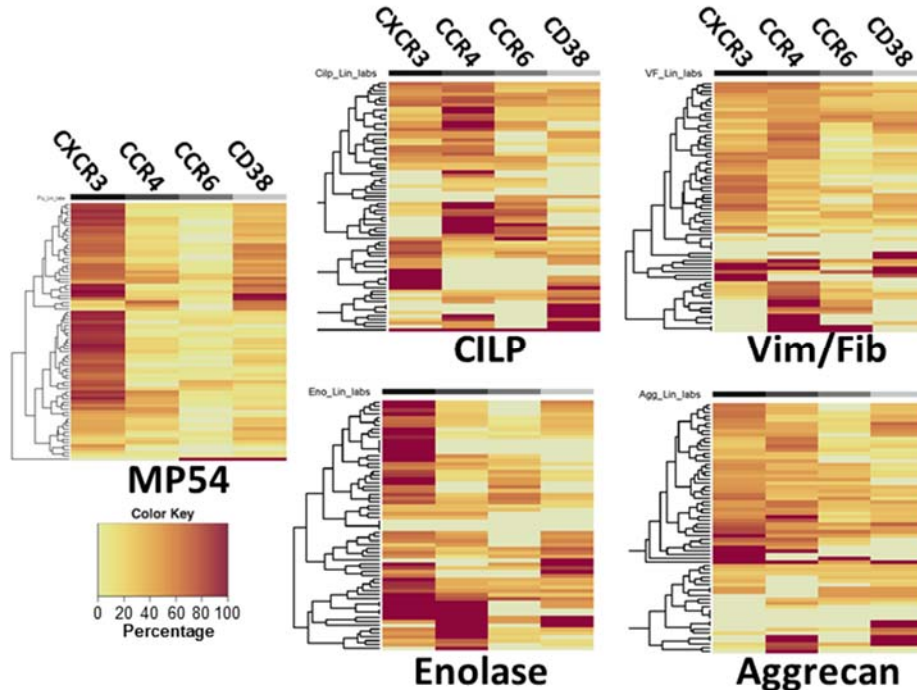
Figure 2. CD4 T cells for citrullinated synovial antigens have heterogeneous phenotypes. Heatmaps of percentage of memory+ Tmr+ cells for specific citrullinated antigens showing expression of cell surface chemokine and activation markers from 55 RA subjects. MP54 is an influenza epitope used as a control. A dendrogram indicating the unsupervised hierarchical clustering relationships is shown to the left of each heatmap.

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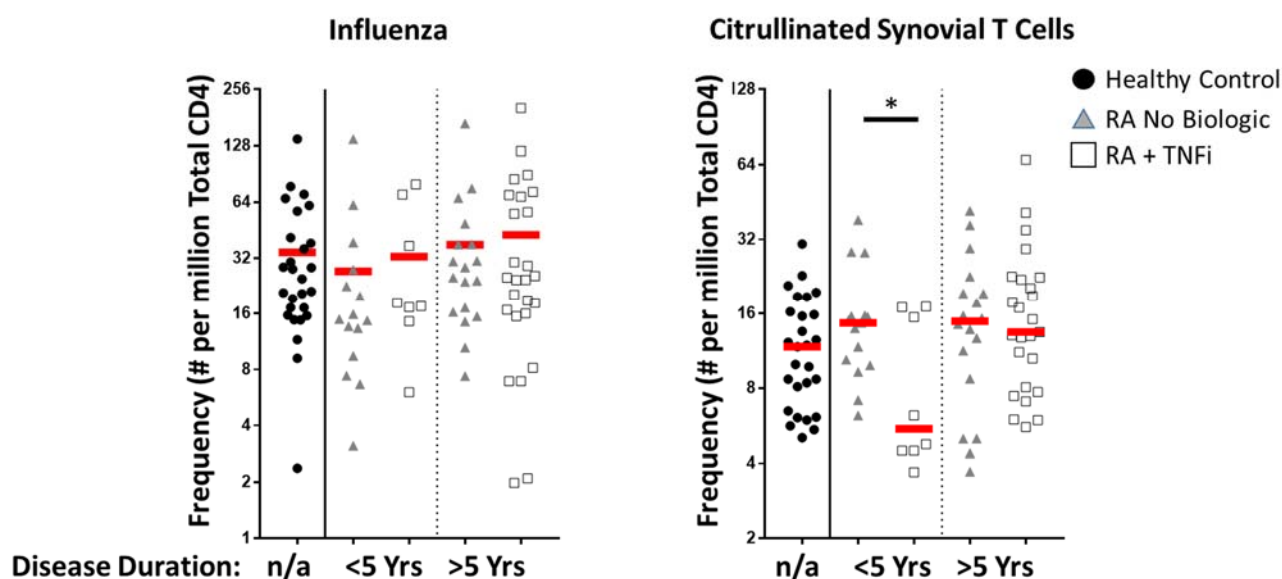
Figure 3. CD4 T cells for citrullinated synovial antigens respond to biologic therapies targeting TNF-alpha in RA subjects with disease duration less than five years. TNF inhibitors have no effect on the frequency of influenza specific T cells in RA subjects. In contrast, the frequency of antigen-specific T cells targeting citrullinated synovial epitopes are significantly decreased in RA subjects on TNF inhibitors within five years of diagnosis. Welch's unpaired t test *p-value <0.05

Table 1: Patient Enrolment for Longitudinal Study's Treatment Group

TREATMENT GROUP (RECRUITMENT GOAL)	TIME POINT 1 (PRE-Tx)	TIME POINT 2 (2-6 MONTHS POST Tx)
NON-BIOLOGIC MTX[1] + DMARDS[2] (12 SUBJECTS)	14	10
BIOLOGIC ANTI-TNF[3] (12 SUBJECTS)	23	20
BIOLOGIC ORENCIA[4] (12 SUBJECTS)	11	9
BIOLOGIC ACTEMRA[5], RITUXAN[6] & XELJANZ[7] (12 SUBJECTS)	15	11

1. Methotrexate; 2. DMARDs: Leflunomide, Azathioprine, Sulfasalazine, and Hydroxychloroquine; 3. Anti-TNF: Adalimumab (Humira); Etanercept (Enbrel); Golimumab (Simponi); and Infliximab (Remicade); 4. CTLA-4Ig: Abatacept; 5. Anti-IL6R: Tocilizumab; 6. Anti-CD20: Rituximab; 7. JAK inhibitor: Tofacitinib.

Appendix II: Abstracts and Meeting Program

1. **2017 FOCIS Annual Meeting Abstract Number: T.18.**
   Identification and Functional Characterization of T cell Reactive to Citrullinated Tenascin-C in HLADRB1* 0401-Positive Rheumatoid Arthritis Patients
   Jing Song, Cliff Rims, David Arribas-Layton, Eddie James and Jane Buckner
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The paracaspase MALT1 is a key player in the activation of lymphoid, myeloid and mast cells, indicating MALT1's crucial role in innate and adaptive signaling. Therefore, MALT1 is regarded a promising target for the treatment of autoimmune diseases and defining its role in the pathogenesis of rheumatoid arthritis (RA) is a critical first step.

To unravel MALT1's role in RA, we initially assessed MALT1-activation in mice challenged with collagen-induced arthritis (CIA), the prototype model for RA. We then sought to address MALT1's role in the pathogenesis of RA by subjecting MALT1-deficient mice to the CIA model. To determine the importance of MALT1 in T-cells, CIA was additionally induced in CD4-specific MALT1-deficient mice. Finally, the effect of MALT1-deletion on bone homeostasis was assessed by measuring bone density by μCT-analysis of the tibiae and by a three-point bending test of the femurs.

We provide evidence that MALT1 is activated in RA and plays a crucial role in its pathogenesis since MALT1-deficent mice were completely protected against CIA. This protection was additionally observed in CD4-specific MALT1-deficient mice, indicating that the selective ablation of MALT1 in CD4-positive cells is sufficient for the observed resistance. Paradoxically to the protective effect of MALT1-deletion on inflammation, we show that MALT1-deficiency negatively influences bone density at steady state.

Altogether, our data provide evidence for a dual role of MALT1 in arthritis, showing a protective effect of its deletion on the inflammatory aspect and a negative effect on bone homeostasis.

T.18. Identification and Functional Characterization of T cell Reactive to Citrullinated Tenascin-C in HLA-DRB1*0401-Positive Rheumatoid Arthritis Patients

Jing Song, Cliff Rims, David Arribas-Layton, Eddie James and Jane Buckner

Benaroya Research Institute at Virginia Mason, Seattle, WA

Anti-citrullinated protein antibodies (ACPAs) are a hallmark of rheumatoid arthritis (RA) and target a number of synovial and inflammation associated proteins. Antibodies against Tenascin-C, an extracellular matrix protein, have been observed in ACPA+ RA patients and a citrullinated peptide was recently identified as their major target. Our aim was to determine whether T cell responses against cit-tenascin-C are present in subjects with RA. We utilized an algorithm to predict 64 possible HLA-DRB1*0401 restricted epitopes within tenascin-C based on its binding motif. These peptides were tested in a binding assay, identifying 10 citrullinated peptides that bound with moderate to high affinity. We next performed *in vitro* assays, expanding PBMC obtained from HLA-DRB1*0401+ patients and staining with HLA class II tetramers, confirming 6 of these peptides as immunogenic. We utilized tetramers to directly stain cit-tenascin-C specific T cells and observed that tenascin-C specific cells were readily visualized in the peripheral blood of HLA-DR*0401+ patients. To further investigate the specificity of tenascin-C specific T cells, we isolated a T cell clone from an RA patient using *ex vivo* single cell sorting. The expanded clone remained tetramer positive and proliferated in response to a citrullinated tenascin-C peptide (1012-1026 modified at amino acids 1014 and 1016). These results demonstrate that T cells that recognize citrullinated tenascin-C peptides are present in HLA–DRB1*0401+ RA patients. We expect that further characterization of cit-tenascin-C specific T cells will indicate unique functional characteristics and others that are shared by T cells that recognize conventional RA autoantigens

T.19. Gut-derived TNF as Risk Factor for the Development of Sacroiliac Inflammation

Karlijn Debusschere[1], Heleen Cypers[1], Peggy Jacques[1], Filip Van den Bosch1[3], Donald Souza[2], Maryanne Brown[2], Devan Dove[2], Gerald Nabozny[2], Alexander Klimowicz[2] and Dirk Elewaut[1]

[1]VIB (the Flanders Institute for Biotechnology) and Ghent University, Gent, Belgium, [2]Boehringer Ingelheim, Ridgefield, CT,

An intruiging link exists between gut and joint inflammation in spondyloarthritis (SpA), with about 50% of SpA patients having subclinical gut inflammation, which represents a risk factor for development of Crohn's disease, sacroiliitis and

Cross Sectional Analysis of Citrullinated-Synovial Antigen-Specific CD4+ T Cells in an RA Cohort Demonstrates Antigen Based Differences in T Cell Frequency, Phenotype and the Influence of Immunotherapy

Cliff Rims[1], Sylvia Posso[1], Bernard Ng[2], Jeffrey Carlin[3], Eddie James[4] and Jane H. Buckner[4], [1]Translational Research, Benaroya Research Institute at Virginia Mason, Seattle, WA, [2]Rheumatology, VA Puget Sound Healthcare System, Seattle, WA, [3]Rheumatology, Virginia Mason Medical Center, Seattle, WA, [4]Benaroya Research Institute at Virginia Mason, Seattle, WA

SESSION INFORMATION

Date: Sunday, November 5, 2017

Session Title: T Cell Biology and Targets in Autoimmune Disease

Session Type: ACR Concurrent Abstract Session

Session Time: 4:30PM-6:00PM

Background/Purpose:

The presence of ACPA in RA indicates that an immune response directed toward citrullinated synovial antigens participates in disease development or persistence. Research from our group have identified T cell targets derived from the auto-antigens aggrecan, vimentin, fibrinogen, alpha-enolase, and cartilage intermediate layer protein (CILP). In this study, we visualized peripheral antigen-specific CD4+ T cells using a multiplexed flow-cytometry based HLA class II tetramer assay in a cross-sectional cohort of 80 RA and 30 matched healthy control subjects to understand their relevance to RA disease progression and response to therapy.

Methods:

All subjects were DRB1*04:01. RA subjects were CCP positive and represented a range of characteristics including time from diagnosis, disease activity and treatment at the time of blood draw. Antigen-specific T cells were visualized by directly staining peripheral blood mononuclear cells (PBMC) with multiple tetramers corresponding to different antigens. Frequencies and phenotypic features of antigen-specific CD4+ T cells were assessed for correlation with clinical characteristics.

Results:

Ex-vivo analysis of PBMC revealed an increase in synovial targeted CD4 T cells when compared to matched healthy DRB1*04:01 subjects. When analyzed by individual antigen CD4 T cells,

aggrecan, vimentin and fibrinogen were increased in RA, and by contrast cartilage-intermediate-layer-protein (CILP) and enolase specific T cells were reduced in comparison to healthy subjects, suggesting that the characteristics of the CD4+ T cells response to synovial epitopes may be unique to antigen specificity.

Within this patient cohort we found a lower frequency of synovial specific T cells in individuals on TNF therapies sampled within 5 years of diagnosis. These differences were most pronounced in the CD4 T cells specific for aggrecan, vimentin and fibrinogen, and showed alterations in chemokine receptor and activation marker expression in the treated group.

Ongoing studies will determine if frequency, phenotype and specificity of synovial specific CD4 T cells correlate directly with disease duration, therapeutic duration, and clinical diagnostic values such as level of RF, CCP, CRP, and disease severity.

Conclusion:

We have shown that a multiplexed tetramer assay can define the breadth and character of the T cell response to synovial antigens. Characterizing a relatively large cohort of subjects, we demonstrate differences in the phenotype and frequency of the T cells that respond to a diverse set of synovial antigens thought to be important targets in RA. In particular, we show that synovial specific T cell frequency is influenced by therapeutic interventions. Better understanding the interplay of antigen specificities and phenotypes in RA is vital to understanding disease pathogenesis, response to therapy and ultimately developing antigen specific therapies.

International Forum for RA - *Preliminary program*

Saturday, September 23, 2017

14:00 – 14:30	Welcome and introduction to the history of and purpose of IFRA
	Lars Klareskog, Zhanguo Li, Kazuhiko Yamamoto

Session 1: The good aspects of the situation today and how we arrived here

14:15 – 14:45	*Chairs: Zhanguo Li, Kazuhiko Yamamoto*
	From ideas in research to clinical benefits. The long term perspectives
	Sir Ravinder Maini, Kennedy Institute, University of Oxford

Session 2: The unresolved clinical and scientific problems of today – and why are they unresolved?

	Chairs: Josef Smolen, Medical University of Vienna and representative from PARE/EULAR
14:50 – 15:15	Patients' needs and current scientific approaches to RA; Not always aligned.
	Johan Askling, Karolinska Institutet, Stockholm
15:15 – 15:40	Clinical unresolved problem as seen from "real life" experience and registries
	Merete Hetland, Copenhagen University
15:40 – 16:05	Scientific unresolved problems in science
	Chris Buckley, University of Birmingham
16:05 – 16:35	Discussions on need for novel solutions to adess the most important unresolved questions
	Discussion led by chairpersons
16:35 – 17.00	***Afternoon break with tea and coffee***

Session 3 Why do we get RA (1st part); What can we learn from genetic epidemiology and genomics?

	Chairs: Mike Brenner, Harvard Medical School and TBA
17:00 – 17:25	"Those who have cohorts are kings, those who have biomarkers are beggars". On needs and benefits of genetics
	Peter Gregersen, Feinstein Institute, New York, NY
17:25 – 17:50	What can studies on environment and life style tell us?

	Saedis Saevarsdottir, Karolinska Institutet, Stockholm
17:50 – 18:15	What can a combination of genetics, environment, epigenetics and omics tell us about molecular pathways towards RA?
	Soumya Raychadhuri, Harvard Medical School, Boston, MA
18:15 – 18:40	Additional contributions
	TBA
18:40 – 19:00	Discussions on strategic thinking concerning combinations of cohorts and biomarkers for future design of studies on pathogenesis, prevention and therapy
	Discussion introduced and led by chairpersons
19:00 – >	*Reception at Aula Medica*

Sunday, September 24, 2017

Session 4: Why do we get RA? (2nd part) The longitudinal course of RA

	Chairs: Anna Rudin, Gothenburg University and Mike Holers, University of Colorado at Denver
8:30 – 8:55	From triggering to targeting; the longitudinal course of seropositive RA
	Anca Catrina, Karolinska Institutet, Stockholm
8:55 – 9:20	Autoantibodies and their glycosylation during emergence of RA
	HU Scherer, Leiden
9:20 – 9:45	ACPAs, NETs and inflammation. New light on neutrophils
	Mariana Kaplan, NIH, Bethesda, MA
9:45 – 10:20	Pathobiology of Rheumatoid Arthritis: Towards a Molecular Definition and Precision Medicine
	Cos Pitzalis, London
10:20 – 10:40	Discussions on strategies to understand the early stages of RA
	Discussion introduced and led by chairpersons
10:40 – 11:05	*Morning break with coffee and fruits*

Session 5: Why do we get RA? (3rd part): A major question: Can RA (or subsets of RA) be triggered by infectious agents?

	Chairs: Rene Toes, Leiden University Medical Center and Solbritt Rantapää-Dahlqvist, Umeå
11:05 – 11:30	The bacterial way towards RA 1: The porphyromonas and PAD pathway
	Karin Lundberg, Karolinska Institutet, Stockholm

11:30 – 11:55	The bacterial way towards RA 2: The Aggregatibacter and hypercitrullination way. **Felipe Andrade, Johns Hopkins, Baltimore, MD**
11:55 – 12:20	The microbiome way **TBA**
12:20 – 12:45	Discussions *Discussion introduced and led by chairpersons*
12:45 – 14:00	*Lunch*

Session 6: Adaptive vs. Innate immunity and mesenchymal functions as driving forces in RA

	Chairs: Andy Cope, King's College London and Steffen Gay, University of Zürich
14:00 – 14:25	Role of specific B and T cells immunity in the initiation of RA **Vivianne Malmström, Karolinska Institutet, Stockholm**
14:25 – 14:50	The B cell repertoire in RA **Dan Mueller, University of Minnesota**
14:50 – 15:35	Effects of monoclonal antibodies from RA patients on innate functions of synovial cells **Bill Robinson, Stanford University, CA**
15:25 – 15:50	On innate immune and mesenchymal functions **TBA**
15:50 – 16:15	*Afternoon break with coffee*

Session 7: adaptive immunity vs innate immunity and mesenchymal functions in RA (continued

16:15 – 16:40	T cell reactivities and regulation in RA **Kazuhiko Yamamoto, University of Tokyo**
16:40 – 17:05	Genetics, T cell specificity and T cell regulation in RA **Jane Buckner, Benaroya Research Institute, Seattle, WA**
17:05 – 19:30	How can adaptive immune functions influence mesenchyme in RA ? **Caroline Ospelt, University of Zürich**
17:30 – 19:30	*Posters, discussion clubs and other informal meetings with wine and*

cheese

Monday, September 25, 2017

Session 8: The systemic features of RA; focus on pain and cognition

	Chairs TBA
8:30 - 8:55	Pain and cognition in RA, Which are the central mechanisms? **Georg Schett, Erlangen University Clinic**
8:55 – 9:20	Longitudinal development of pain in RA **Jon Lampa, Karolinska Institutet, Stockholm**
9:20 – 9:45	Fatigue in RA; how to evaluate? **TBA**
9:45 – 10:10	Discussions *Discussion introduced and led by chairpersons*
10:10 – 10:35	*Morning break with coffee*

Session 9: Mechanisms of pain, fatigue and more

10:35 – 11:00	Antibody-mediated pain **Camilla Svensson, Karolinska Institutet, Stockholm**
11:00 – 11:25	Interactions between nerve system and inflammation in inflammatory diseases **speaker to be confirmed**
11:25 – 11:50	Mechanisms of chronic pain **TBA**
11:50 – 12:15	Additional contributions **TBA**
12:15 – 12:40	Discussions *Discussion introduced and led by chairpersons*
12:40 – 14:00	*Lunch*

Session 10 New ways of understanding the broad spectrum of the patient's problems in RA

	Chairs: Representatives from Asia and from patients' organizations
14:00 – 14:25	What does really matter for patients and in therapeutic trials **Iain McInnes, University of Glasgow**
14:25 – 14:50	How to understand and measure "subjective symptoms" that matter for the patient ? **Karim Raza, University of Birmingham**

14:50 – 15:15	What matters for patients in China?
	Zhanguo Li, Beijing
15:15 – 15:40	Discussions
	Discussion introduced and led by chairpersons
15:40 – 16:15	*Afternoon break with coffee*

Session 11: New ways of understanding the individual's most urgent problems and how they change over time; What role for e-health

16:15 – 16:40	*Chairs: Gerd Burmester, Charité, Berlin and TBA*
	TBA
	Will Dixon, University of Manchester
16:40 – 17:05	Meeting the patients early with an app: What options and what obstacles?
	Sofia Ernestam, Karolinska Institutet
17:05 – 17:30	What will happen with "patient reported outcomes" in the future?
	Tore Kvien, Oslo University
17:30 – 18:15	Abstracts and/or Discussions
18:30	*Leave for City Hall (tentative)*

Tuesday, September 26, 2017

Session 12: New targets for prevention and therapy – adaptive and innate immunity again

8:30 – 8:55	Tolerance therapies – will they become real?
	Ranjeny Thomas, University of Queensland
8:55 – 9:20	Tolerizing T cells
	Rikard Holmdahl, Karolinska Institutet
9:20 – 9:45	On PAD inhibition. Specific therapies do not need to be antigen specific
	TBA
9:45 – 10:15	One or two abstracts and discussions
10.15 - 10:40	*Morning break with coffee*

Session 13 Future trials for prevention and curative treatments; Which strategies?

10:40 – 11:05	New smart trial designs **John Isaacs, Newcastle University**
11:05 – 11:30	Trials for prevention: How to do it? **Tom Huizinga, Leiden**
11:30 – 11:55	Therapy is not only medicines. How to accomplish and measure effects of change of life style and environment? **Jill Norris, Denver**
11:55 – 12:20	One or two abstracts
12:20 – 13:30	Final Discussions: Ways forward
13:30	Lunch and Departures

OTHER SUPPORT

BUCKNER, Jane H

5 U01 AI101990-04 (Buckner)	07/01/2012 – 06/30/2018	2.04 calendar
NIH/NIAID	$2,008,657	

Defining the role of altered cytokine signaling pathways on autoimmunity
We pose the hypothesis that in autoimmune individuals, enhanced phosphorylation of STAT3 and diminished phosphorylation of STAT5 establish a functional program biasing immune responses towards a skewed, pro-autoimmune profile. Award includes an administrative supplement in support of the Cooperative Study Group for Autoimmune Disease Prevention (CSGADP). The CSGADP goals is to halt the development of autoimmune disease prior to clinical onset by means other than global immunosuppression, the CSGADP will support collaborative projects, innovative pilot and feasibility projects, and development of reagents and resources. The Infrastructure and Opportunities Fund (IOF) of the CSGADP will facilitate the mission.

1 DP3 DK104466-01 (Buckner)	09/19/2014 – 08/31/2018	0.18 calendar
NIH/NIDDK	$145,683	

Investigating the role of IL-6 signaling in Teff resistance and T1D development
The focus of this project is enhanced responsiveness to IL-6 and or IL-21 predates the development of T1D and contributes to the development of Teff resistance. Additionally, we will examine the hypothesis that the combination of enhanced IL-6(pSTAT3) and blunted IL-2(pSTAT5) responses result in an increase in pathogenic CD4 T cells and impaired Treg development and function resulting in the progression to T1D.

1 R01AR065952-01A1 (Hawkins)	03/01/2015 – 01/31/2019	0.24 calendar
NIH/NIAMS	$4,109	

Functional validation of Rheumatoid Arthritis-associated distal related regulatory SNPs
This proposal is to functionally validate distal regulatory SNPs associated with rheumatoid arthritis.

1 UC4 DK097835-01 (Krischer)	09/30/2015 – 06/30/2020	0.60 calendar
NIH/NIDDK	$14,800	

NIDDK T1D TrialNet Data Coordinating Center | TrialNet Core Biomarkers and Mechanisms Panel (BMP)
Members of the TrialNet Core BMP are charged with development and execution of a strategic plan for mechanistic and biomarkers research within TrialNet towards its mission of Type 1 Diabetes prevention.

5 UM1 AI109565-04 (Nepom)	08/01/2014 – 01/31/2019	0.60 calendar
Immune Tolerance Network	$38,399	

Dr. Buckner is Co-Protocol Chair of this trial with Dr. Carla Greenbaum. ITN Clinical Trial Protocol Chairs have overall responsibility for the conduct of the study, including oversight of all scientific, reporting, and financial matters. They will oversee recruitment activities, patient care management, and participate in monthly study teleconferences. Under the guidance of the ITN members, Protocol Chairs also interact with regulatory agencies, as needed, for matters regarding this clinical trial.

2 U01 AI101981-06 (Holers)	07/001/2017 – 06/30/2022	0.60 calendar
NIH/NIAID	$125,000	

Prevention Center U01: Early targets for antigen-specific tolerance induction in Preclinical Rheumatoid Arthritis
The goal of this project is to characterize the antigen reactivity of dual IgA/IgG plasmablasts and mucosally produced IgA/IgG, determine which citrullinated antigens and microbial species are recognized, understand how clonotypes and effector function of individual citrullinated antigen-specific clonal families in B cells evolve to be increasingly pathogenic, and characterize CD4+ T cells that recognize citrullinated and novel antigens.

1R01AI132774-01 (Buckner)	07/01/2017-06/30/2022	1.80 calendar
NIH/NIAID	$490,997	

Mechanisms of IL-6 mediated T cell pathogenesis in autoimmunity

The goal of this study is to test the hypothesis that elevated membrane bound IL-6 receptor expression leads to altered T cell fate and function resulting in pathogenic autoreactive T cells due to changes in the magnitude and balance of STAT1 and STAT3 phosphorylation.

2016PG-T1D039 (Rawlings)   12/01/2017-11/30/2019   0.24 calendar
Helmsley Foundation $177,000
Development of antigen-specific eTreg therapy in T1D (Stage 2 Studies)
The goal of this project is to develop autologous, pancreatic islet-antigen specific, FOXP3-espression stabilized, engineered regulatory T cell products to treat or prevent type 1 diabetes.

PENDING
R21 (Buckner)   04/01/2018-03/31/2020   0.60 calendar months
NIH $150,000 Year 1 Directs
Exploring novel disease mechanisms in RA linked to HLA class II
The goal of this study is to test the hypothesis that the HLA DRB1*04 and *1001 alleles associated with increased risk of RA promote alterations in T cell fate and function that contribute to the development and progression of RA, independent of antigen specificity.

R01 (Buckner)   07/01/2018-06/30/2023   0.60 calendar months
NIH $445,820 Year 1 Directs
Defining the features of T cell response to tumor and self-antigens as predictors of response to checkpoint therapy
This application is in response to the National Cancer Institute's Provocative Question #8: "What are the predictive biomarkers for the onset of immune-related adverse events (irAE) associated with checkpoint inhibition, and are they related to markers for efficacy?". The application proposes to test the hypothesis that the frequency and phenotype of T cells specific for self-antigens predicts autoimmune irAE, which in turn predicts therapeutic efficacy in some patients.

R01 (Cerosaletti)   07/01/2018-06/30/2023   0.24 calendar months
NIH $457,437 Year 1 Directs
Modulating autophagy and plasmablast differentiation in lupus
The goal of this project is to test the hypothesis that SLE-associated risk variants in BANK1 promote autophagy, leading to increased plasmablast differentiation and/or immunoglobulin (Ig) secretion that contributes to lupus susceptibility and disease.

Ng, Bernard

No changes in other support

<u>**ACTIVE**</u>
NONE

<u>**COMPLETED RESEARCH SUPPORT**</u>

Grant # VA.SCV.1010./000-00.B_N   10/01/2010 – 09/30/2012
South Central VA Health Care Network Research Grants Program
Evaluating the Optimal Use of Traditional Disease-Modifying Drugs of Rheumatic Diseases in the Biologic Era
Brief summary – Since the advent of biological DMARDs, there has been reduced interest in the use of traditional DMARDs. Most recent literature, especially those funded by large pharmaceuticals, focuses on new and expensive drugs. There is little funding and interest to look at optimizing MTX use and the use of triple traditional DMARDs RA therapy. Knowing the trends of prescription behavior of DMARDs will be important to make cost-effective recommendations regarding traditional and biological DMARDs.
Role: Principal Investigator

Grant # CF.HQU.LFP.0110.000-00.B_N10/01/2009   09/30/2010
Houston VA Health Services Research and Development Center of Excellence
Is low or moderate alcohol consumption safe in patients with Rheumatoid Arthritis (RA) on Methotrexate
Brief summary- Methotrexate (MTX) has been used extensively in the treatment of RA since the early 1980s. It is a drug with a high benefit-to-risk ratio compared to many traditional disease-modifying anti-rheumatic drugs (DMARDs). In addition, low cost, convenient weekly dosing and wide availability make MTX a preferred drug over several other DMARDs. It is usually recommended that one should abstain from alcohol while taking MTX because of the fear of liver toxicities. This is a conservative recommendation that is based solely on expert opinions because there is no clinical data about the quantity of alcohol that can be safely consumed with MTX. A study commented that more patients refused Leflunomide, another DMARD which abstinence from alcohol is required, when told that they needed to abstain from alcohol. Though it is not unreasonable to assume that alcohol will adversely affect MTX compliance, there are no well-established studies in this area unlike the treatment of HIV and diabetes. If low or moderate alcohol consumption is found to be safe when taking MTX, the compliance for MTX may be improved when patients are told that complete abstinence from alcohol is not required.
Role: Principal Investigator

Grant # (Huston, D.P.)   07/01/2002 – 06/30/2003
GlaxoSmithKline/American Academy of Allergy Asthma and Immunology
Atomic Delineation of the βc Receptor Binding Domain of IL-5
Brief summary –The grant funded a study that tested the hypothesis that the IL-5 domain that engages and/or activates the βc subunit of the IL-5 receptor involves a specific charge field around the Glu13 residue. The corollary hypothesis is that a charge alteration at this site will result in an IL-5 molecule still capable of binding the IL-5Rα subunit but unable to transduce and/or recruit an agonistic signal through the βc subunit. Such molecules should have the potential to be a therapeutic molecular antagonist of IL-5-mediated eosinophilic inflammation.
Role: Research/Clinical Fellow

<u>**OVERLAP**</u>
There is no scientific or budget overlap between these projects. If pending grants are awarded, the percent/calendar month effort will be adjusted accordingly.

LINSLEY, Peter

<u>**ACTIVE**</u>

1 DP3 DK104465-01 (Linsley)	09/25/2014 – 08/31/2018	1.50 calendar
NIH/NIDDK	$640,925	

Determining the molecular basis for different rates of T1D progression
Our goal is to identify molecular and/or cellular signatures in whole blood that characterize non-progressor responses to different therapies and during natural history, and to determine whether these signatures are unique or treatment-specific. From these signatures, we anticipate discovering unique, data-driven insights into immunological aspects of T1D progression.
Role: PI

1 DP3 DK110867-01 (Linsley)	08/01/2016-07/31/2018	1.51 calendar
NIH/NIDDK	$297,376	

Single Cell Transcriptome Analysis of Islet Antigen Reactive Memory CD4+ T Cells in Established TID
We are using cutting edge now cytometry and systems biology approaches to identify single cell transcriptome signatures in islet antigen reactive memory CD4+ (IARM-CD4) T cells from subjects with established TID and healthy controls. We anticipate discovering unique, data-driven insights into immunological aspects of T1D progression. This study will also provide information regarding the feasibility, lime, cost, effect size and variability needed to design appropriate follow on studies to rigorously evaluate IARM-CD4 T cells as new biomarkers and therapeutic targets.
Role: PI

5 R01 AI108839-04 (Wambre)	07/01/2014 – 06/30/2019	.076 calendar
NIH/NIAID	$250,000	

Induction and signature of pathogenic T cells in allergy
The purpose of this study is to identify a CD4+T cell signature for allergic diseases resulting from a comprehensive understanding of the mechanisms associated with the pathogenesis of allergic disease and peripheral tolerance to allergens.
Role: Co-I

3-SRA-2014-315-M-R (James, E)	09/01/2014 – 08/31/2018	0.9 calendar
JDRF	$416,773	

Immune effector and regulatory balance as a predictor for preserved beta cell function in subjects with established T1D
This project will investigate the central hypothesis that T cell effector and regulatory balance represents a key mechanism that determines the persistence of C-peptide in established T1D.
Role: Faculty Collaborator

W81XWH-15-1-003 (Buckner, JH)	12/10/2014 – 12/09/2018	1.2 calendar
Department of Defense – USAMRAA	$247,619	

In Depth Analysis of Citrulline-specific CD4 T Cells in Rheumatoid Arthritis
The focus of this project is to test the hypothesis that cit-specific CD4 T cells present in RA patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identify novel therapeutic targets for the treatment of RA.
Role: Co-I

1 DP3 DK106909-01 (Kwok)	09/01/2015–08/31/2018	0.48 calendar
NIH/NIDDK	$1,290,209	

Phenotypic Analysis of Islet Antigen-specific Effector T cells in Pre-diabetic Subjects
The aims are to test the following hypotheses: 1. that an increase in the frequency of recently activated auto-reactive CD4+ and CD8+ T cells precedes the onset of clinical diabetes; 2. that prediabetic subjects that progress to T1D acquire an expanded TCR repertoire of islet antigen specific T cells and that those T cells

exhibit a distinct transcript signature; and 3. that progression toward T1D is accompanied by an imbalance between Treg function and effector T cell responsiveness and by periods of active beta cell destruction.
Role: Co-I

2-SRA-2016-307-S-B (Linsley)	09/01/2016 – 08/31/2018	1.2 calendar
JDRF	$225,910	

Triggering EFFECTOR T cell exhaustion to enhance tolerance to autoantigens induced by TCR-targeted agents in T1D
We aim to further characterize the distribution of MCD8TEK cells, their T cell exhaustion phenotype(s), and their relationship to islet autoreactive T cells. Our goals are to overcome obstacles to successful therapy with teplizumab; identify new biomarkers for successful therapy; and to exploit T cell exhaustion as a novel mechanism for treatment of T1D.
Role: PI

5UM1AI109565-04 (Nepom)	2/1/17-1/31/18	1.8 calendar
NIH/NIDDK	$344,581	

EP178: A single cell RNA-seq analysis approach to characterize antigen-specific CD8 T cells ITN027AI AbATE
The objective is to optimize and apply a single cell RNA-sequence analysis approach to characterize antigen-specific CD8 T cells; profiling disease relevant cells at baseline, after therapy and comparing with profiles obtained by interrogating broader T cells subsets.
Role: Subaward CO-I

1 R01 AI132774-01 (Buckner)	07/18/2017-06/30/2021	0.6 calendar
NIH/NIAID	$495,023	

Mechanisms of IL-6 mediated T cell pathogenesis in autoimmunity
The IL-6 signaling pathway is dysregulated in autoimmunity in part through increased expression of the membrane bound IL-6R(mbIL-6R). The central hypothesis for the proposed studies is that elevated mbIL-6R expression leads to altered T cell fate and function resulting in pathogenic autoreactive T cells due to changes in the magnitude and balance of STAT1 and STAT3 phosphorylation.
Role: Co-Investigator

5UM1AI109565-04 (Nepom)	02/01/2017 – 01/31/2018	0.77 calendar
NIH/NIAID	$31,271	

Single Cell Transcriptome Analysis of Islet Antigen Reactive Memory CD4+ Cells
The aims are to test the following hypotheses: 1) Determine the frequency, stability and specificity of expanded TCR clonotypes in IARM-CD4 T cells. 2) Validate the specificity of expanded TCR pairs. 3) Characterize the transcript phenotypes of IARM-CD4 T cells and determine the generality, stability and clonotype specificity of these signatures.
Role: Sub Award PI

1 U19 AI135817-01 (Kwok WW)	01/01/2018 - 12/31/2022	1.2 calendar
NIH	$50,667	

Allergen T cell epitopes and phenotypes in peanut allergy and immunotherapy
The overall aim of this center is to understand the role of peanut epitope-specific T-cell responses in the pathogenesis and treatment of peanut allergy by utilizing peanut T cell epitope-specific tetramer reagents and other novel assays. New knowledge gained in this proposal should facilitate the practice of precision medicine and the development of novel intervention in treating subjects with peanut allergy.
Role: Project PI

Collaborative Research Agreement		
Bristol-Myers Squibb Company	12/30/2016-12/29/2018	
Confidentiality Agreement in Place		

Collaborative Research Agreement	12/30/2016-12/29/2018	
Celgene Company		

Confidentiality Agreement in Place

<u>PENDING</u>

1R01AI139606-01 (Linsley)   07/01/2018 – 06/30/23   1.8 calendar
NIH/NIAID $411,360
Single-cell RNA sequencing reveals expanded clones of myelin-reactive CD4+ T cells in peripheral blood of subjects with Multiple Sclerosis (MS)
Our goal is to elucidate molecular mechanisms that drive clonal expansion of myelin-reactive CD4+ T cells in MS in order to increase fundamental understanding of disease progression, and aid in development of proximal mechanism-based biomarkers and therapies for MS.
Role: PI

1R01CA231226-01 (Buckner, Linsley, Wambre)   07/01/2018 – 06/30/23   3.0 calendar
NIH/NCI $445,820
Defining the features of T cell response to tumor and self-antigens as predictors of response to checkpoint therapy
Immune checkpoint inhibitors are drugs that unleash the immune system to attack cancer cells. Although they have been shown great efficacy, they do not work in all patients and often have serious side effects. The goal of this project is to identify biomarkers that predict immune checkpoint inhibitor efficacy and/or side effects.
Role: MPI

1R21AR073508-01 (Buckner)   04/01/2018-03/31/2020   0.60 calendar
NIH $150,000
Exploring novel disease mechanisms in RA linked to HLA class II
The goal of this study is to test the hypothesis that the HLA DRB1*04 and *1001 alleles associated with increased risk of RA promote alterations in T cell fate and function that contribute to the development and progression of RA, independent of antigen specificity.
Role: CO-I

<u>OVERLAP:</u>

NONE

JAMES, Eddie A.

<u>ACTIVE</u>

2-SRA-2018-551-S-B (James)	10/01/2017 – 09/31/2019	1.8 calendar
JDRF	$227,273	

Integrated Analysis of T cell Responses to Modified Beta Cell Antigens
We seek to validate a multi-color tetramer assay to broadly characterize CD4+ T cell responses directed against diverse modifications and antigens in subjects with human T1D and to demonstrate that monitoring such responses provides a useful biomarker that can be used as in immune correlate for patient stratification and predict the risk of progression and beta cell loss after diagnosis.
Role: PI

5UM1AI109565-04 (Nepom J)	02/01/2017 – 01/31/2018	0.18 calendar
ITN	$344,581	

EP178: A single cell RNA-seq analysis approach to characterize antigen-specific CD8 T cells ITN027AI AbATE
The objective is to optimize and apply a single cell RNA-sequence analysis approach to characterize antigen-specific CD8 T cells; profiling disease relevant cells at baseline, after therapy and comparing with profiles obtained by interrogating broader T cells subsets.
Role: Subaward CO-I

5 UM1 AI109565-04 (Nepom)	02/01/17 – 01/31/18	0.23 calendar
NIH/NIAID	$464,703	

EP201: T cell exhaustion signatures in Ag Specific cells in T1D patients with EBV reactivation during anti-CD3 treatment
This project will use tetramer sorting technology to isolate beta cell and EBV Antigen specific CD8 T cells from EBV virus positive and EBV virus negative subjects from the AbATE study. Our aim is to compare cellular phenotype, gene expression profile and epigenetics in order to understand if T1D or EBV are driving the exhaustion phenotype that is associated with anti-CD3 treatment.
Role: Subaward PI

2 R01 DK081166-06A1 (Haskins)	04/01/2016 – 03/31/2021	0.60 calendar
NIH	$76,459	

Hybrid Peptides as Antigens for Diabetogenic CD4 T Cells
The goal of this project is to test the hypothesis that CD4+ T cell responses to hybrid insulin peptides are relevant in human T1D
Role: Subaward PI

1 DP3 DK106909-01 (Kwok)	09/01/2015 – 08/31/2018	0.64 calendar
NIH/NIDDK	$419,653	

Phenotypic analysis of islet antigen-specific effector T cells in pre-diabetic subjects
The aims are to test the following hypotheses: 1. that an increase in the frequency of recently activated auto-reactive CD4+ and CD8+ T cells precedes the onset of clinical diabetes; 2. that prediabetic subjects that progress to T1D acquire an expanded TCR repertoire of islet antigen specific T cells and that those T cells exhibit a distinct transcript signature; and 3. that progression toward T1D is accompanied by an imbalance between Treg function and effector T cell responsiveness and by periods of active beta cell destruction.
Role: CO-I

2-SRA-2015-107-Q-R (James)	08/01/2015 – 07/31/2018	1.02 calendar
JDRF	$107,496	

Validation of an improved HLA class I Combinatorial Multimer Assay
The goal of this project is to validate an improved combinatorial T cell assay as an effective biomarker to predict risk of imminent loss of residual insulin secretion in subjects with type 1 diabetes.
Role: PI

W81XWH-15-1-0003 (Buckner)   12/10/2014 – 12/09/2017   1.08 calendar
Department of Defense $248,540

In-Depth Analysis of Citrulline-Specific CD4T Cell in Rheumatoid Arthritis

The goal of this project is to test the hypothesis that cit-specific CD4 T cells present in RA patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identity novel therapeutic targets for the treatment of RA.
Role: CO-I

3-SRA-2014-315-M-R (James)   09/01/2014 – 12/31/2018   1.20 calendar
JDRF $454,264

Immune effector and regulatory balance as a predictor for preserved beta cell function in subjects with established T1D

This project will investigate the central hypothesis that T cell effector and regulatory balance represents a key mechanism that determines the persistence of C-peptide in established T1D.
Role: PI

5 U01 AI101981-06 (Holers)   07/05/2012 – 06/30/2022   0.60 calendar
NIH-NIAID $125,000

Prevention Center: Early targets for antigen-specific tolerance induction in preclinical rheumatoid arthritis

The goal is to identify novel synovial T cell epitopes in RA, and develop HLA class II tetramers to detect autoreactive T cells. The data generated from these studies will be vital in the development of tools to predict the risk of RA and will also yield information that will assist in the development of treatments to prevent RA.
Role: Subaward PI

2-SRA-2018-482-S-B (James)   10/01/2017 – 01/31/2019   0.12 calendar
JDRF $22,724

Cooperative validation of a multiplex class II HLA multimer assay

This biomarker validation proposal seeks to test the technical precision and utility of a multicolor class II multimer assay and to validate its suitability for monitoring aspects of beta cell specific T cell frequency and phenotype in subjects with type 1 diabetes.
Role: PI

2-SRA-2018-551-S-B (James)   10/01/2017 – 09/31/2019   1.8 calendar
JDRF $227,273

Integrated Analysis of T cell Responses to Modified Beta Cell Antigens

We seek to validate a multi-color tetramer assay to broadly characterize CD4+ T cell responses directed against diverse modifications and antigens in subjects with human T1D and to demonstrate that monitoring such responses provides a useful biomarker that can be used as in immune correlate for patient stratification and predict the risk of progression and beta cell loss after diagnosis.
Role: PI

1DP3DK111802-01 Rawlings (PI)   09/30/2016 – 06/30/2018   .12 calendar
NIH/NIDDK $280,015

An integrated strategy to define the functional and synergistic impact of TID causal variants

This study expands T1D studies to include variants in TYK2, SH2B3 and IFIHI1 in addition to PTPN22.
Role: Co-Investigator

PENDING

1R21AI35780-01A1 (James)   07/01/2018 – 06/30/2020   0.3 calendar
NIH/NIAID $150,000

Neutrophil-NET activity and generation of citrullinated antigens as precipitating events in human autoimmunity

This project seeks to characterize neutrophil-NET signatures and citrulline specific CD4+ T cells in subjects at risk of developing T1D to test the hypothesis that appearance of a neutrophil-specific signature correlates with the expansion of citrulline specific T cells, creating an important link between innate and adaptive responses during the natural history of T1D.
Role: PI

OVERLAP
There is no scientific or budgetary overlap at present. If any overlap in calendar month effort occurs as pending grants are awarded, PI effort will be adjusted in accordance with sponsor regulation and institutional policy.